The Amendments

Claims 31 and 39 have been amended to delete "at least one" and to specify the resultant vector has a size greater than 105% the size of wild-type adenovirus. Support is found, e.g., at page 12, lines 24-29.

New claims 43-62 have been added to more clearly specify the claimed invention. Support for new claim 43 is found in the description on page 2, lines 6-10. Support for new claim 44 is found on page 2, lines 8-10. Support for new claim 45 is found on page 2, lines 11-12. Support for new claims 46-47 is found on page 2, lines 21-24 and on page 4, lines 11-21. Support for new claims 48-49 is found on page 4, lines 32-33. Support for claim 50 is found on page 4, line 34. Support for claim 51 is found on page 4, line 29. Support for claim 52 is found on page 4, line 24. Support for claim 53 is found on page 4, line 27. Support for claim 54 is found on page 5, lines 1-3. Support for claim 55 is found on page 4, line 26. Support for claim 56 is found on page 4, line 25. Support for claims 57-62 is found on page 5, lines 5-8. No new matter has been added.

In response to the Examiner's objection, Applicant has requested the deletion of Figs. 7-10 with replacement by enclosed corrected Figs. 7-10. Applicant has withdrawn Figs. 11 and 12 as inadvertent duplications of Figs. 13 and 14, respectively. The remaining figures are renumbered consecutively to account for the withdrawal of the two figures (Figs. 11 and 12).

The specification has been amended to refer to the correctly renumbered figures. No new matter has been added.

The Rejection under 35 U.S.C. §112

Claims 31, 32 and 39-42 have been rejected under 35 U.S.C., second paragraph, as indefinite with respect to the recitation of "at least one heterologous nucleotide sequence." The claims have been amended to specify "a heterologous nucleotide sequence." This amendment is believed to overcome the rejection.

The Rejections under 35 USC 103

Claims 1, 2, 4, 25-32 and 39-42 have been rejected as obvious over either Callebrant et al. (Coronaviruses 1994) or Torres et al. (Journal of Virology 1996) and either Kleiboeker (Virus Research 1994) or Reddy et al. (Virus Research 1996). This rejection is respectfully traversed.

Broadly, the applicants teach insertion of at least one heterologous nucleotide sequence in a non-essential region of the porcine adenovirus genome to form stable recombinant porcine adenovirus vectors suitable for use in the preparation of vaccines. The size of the recombinant porcine adenovirus vectors used herein, having a genome size greater than 105% of wild type genome, exceeds the previously-known maximum limit for construction of a stable recombinant. These stable recombinant vectors are capable of generating and/or optimizing an immune response so as to provide or enhance protection against infection in pigs.

In support of the invention, the applicants have specifically demonstrated the insertion of various heterologous nucleotide sequences such as (nonexclusively) swine fever virus gene (gp55), porcine granulocyte-colony stimulating factor gene (G-CSF) and granulocyte/macrophage-colony stimulating factor gene (GM-CSF) in various insertion sites within non-essential regions of the right hand end of the porcine genome corresponding to approximately map units 80 to 100 of the genome to produce stable recombinants having a DNA length exceeding the previously-known maximum limit for construction of a stable recombinant.

The invention must be considered in the light of the state of the art as it existed at the priority date of the invention. At that time, porcine adenoviruses had not been examined in detail, with little work published on the characterization of the porcine adenovirus genome. Most published work related to human adenoviruses. From this work, it was understood that recombinant mammalian adenoviruses could accommodate up to 105% of the wild type genomic length, but that genomic lengths in excess of this were inadequately able to be packaged or extremely unstable. It was not expected that an insertion of foreign DNA, which increased the genome size to greater than 105% of wild type, would be stable.

In support of this, we refer the Examiner to the specification at page 12, lines 24-29, referring to Bett, A.J., Prevec, L. and Graham, F.L. from the Journal of Virology 1993 Oct; 67(10) 5911-21(Abstract attached hereto as Exhibit A) entitled "Packaging capacity and stability of human adenovirus type 5 vectors". This publication presents data obtained during serial passage of recombinant human type 5 adenoviruses in tissue culture. The data demonstrates that when the size of the recombinant genome exceeded 105% of the wild type genome, growth of the vector is very poor and the vector undergoes rapid rearrangement, resulting in loss of the insert after only a few passages. In contrast, vectors with inserts resulting in a net genome size of 105% of that of the wild type were shown to grow well and were relatively stable. This document teaches that there is a tight constraint on the amount of DNA that can be packaged into adenovirus virions and that exceeding the 105% limit results in an unstable vector with poor growth rate.

The cited references

The Office Action alleges that the invention as claimed is obvious in the light of the disclosures of Callebrant et al or Torres et al. when combined with either Kleiboeker or Reddy et al. More specifically, the Office Action alleges that it would have been obvious to the skilled person to develop a porcine adenoviral vector as suggested in either Kleiboeker or Reddy et al. for the expression of foreign genes to be used as a vaccine in pigs as discussed in either Callebrant et al. or Torres et al. given the broad general knowledge in the art for the production of recombinant adenoviral vectors by inserting genes into the E3 region (and other regions) of the genome.

Callebrant et al.

Callebrant et al. specifically relates to the construction of a recombinant human adenovirus type 5 (Ad5) expressing a fragment of the gpS gene of porcine respiratory coronavirus. The reference does not teach the production of a porcine recombinant adenoviral vector. Callebrant et al. makes reference to the paper of Graham and Prevec 1991, which teaches that the deletion within the E3 region of the Ad5 virus is not essential for replication of the Ad5 virus.

The Office Action states "the E3 region has been found not to be required for replicating adenovirus in tissue culture, [thus] a gene may be deleted or a gene may be inserted into this region

without effecting replication". It is respectfully submitted that this conclusion cannot be drawn in regard to the porcine adenovirus genome (PAV) from a reading of Callebrant et al. Nucleotide sequence analysis and comparison between human Ad5 and PAV3 show that the E3 region of PAV3 is 1.179 kbp whereas the E3 region of human Ad5 is 3.421 kbp, thus the E3 region of PAV3 is 2.242 kbp smaller than the human adenovirus 5. In addition, there is only 26.3% nucleotide identity between the human Ad5 E3 and PAV3 E3 regions. Given the highly significantly different nucleotide sequences it would not have been known or expected that deletion from PAV3 could be carried out and still allow recombinant virus construction or virus replication *in vitro* or *in vivo*.

Torres et al.

Torres et al. discloses a recombinant human adenovirus type 5 (Ad5) vector expressing different antigenic sites of the transmissible gastroenteritis coronavirus (TGEV) spike protein. There is no teaching of the construction of a porcine adenovirus vector or the use of such a vector.

The Office Action alleges that Torres et al. teaches that "human Ad 5 can replicate in porcine cells and these porcine cells have been shown to support the expression of heterologous sequences when inserted into the E3 region [thus] Ad5 was shown to be effective as a live vaccine in pigs." We would respectfully question this analysis made in the Office Action, the conclusions drawn in respect to human Ad5, and the applicability and relevance of these conclusions in relation to porcine adenoviruses.

It is submitted that Torres et al. shows very low levels of virus replication in porcine cells, if any, and does not demonstrate a cytopathic effect (cpe) at all since virus titres could only be determined by transferring 'infected' swine cells back onto susceptible tissue culture 293 cells. All recombinant virus construction described in Torres et al. was carried out using human Ad5 DNA contained within plasmids which were transfected into 293 cells, not swine cells. Nowhere do the authors instruct or demonstrate how to construct a recombinant PAV or attempt to culture PAV.

The only data presented to demonstrate the replication of human Ad5 in swine cells is not conclusive and does not demonstrate productive infection *in vitro*. Since the TGEV spike gene is

under the control of either the SV40 constitutive eukaryotic promoter or the human Ad5 E3 early promoter, expression of the protein product could occur without virus replication once the virus had delivered the gene to the inside of a cell.

Further, the data presented in Torres et al. is not conclusive and does not demonstrate productive infection *in vivo* in either pigs or hamsters. The two recombinant human Ad 5 constructs administered to pigs contained the TGEV spike gene under the control of the E3 promoter and could therefore have been expressed without virus replication during an abortive infection. Due to the exquisite sensitivity of the immune system it is quite possible that enough protein was produced during such an abortive infection to induce neutralizing antibody production. We would also question the validity or relevance of the protection assays presented in Torres et al. The protection assays were carried out as passive transfer of neutralizing antibody. Passive transfer of neutralizing antibody to susceptible animals is not a commercially viable method of protecting from disease. Pigs that were vaccinated with the recombinant human Ad5-TGEV constructs were not directly challenged with TGEV and this is a flaw in the design of the study. A sound study would provide data on the results of a direct challenge with TGEV and it is possible to infer that direct challenge experiments were carried out but were unsuccessful at mediating protection, and thus not supporting the use of human Ad5 recombinants in pigs. Another consideration in the use of human Ad5 recombinants in pigs is the extremely high doses of recombinant needed to induce an immune response. The vaccination regime reported in Torres et al. consisted of three separate doses given by three separate routes on three separate occasions, where each dose was 1×10^9 plaque forming units. This vaccination regime, number of doses and routes of delivery are unacceptable for commercial use and teach against the use of human Ad5 in pigs. In addition, the recombinant virus used to vaccinate pigs was grown in 293 cells and not swine cells.

In light of the foregoing it is submitted that Torres et al. does not support the Examiner's claim that Ad5 was shown to be effective as a live vaccine in pigs. Following from this, it cannot be concluded that PAV would be effective as a live vaccine in pigs.

We would also draw to the Examiner' attention a related publication by Torres et al, Virology 213, 503-516 (1995) entitled, "Induction of Antibodies Protecting against TGEV by Recombinant Adenovirus Expressing TGEV Spike Protein" (of record). This paper also reports on the construction of recombinant human adenovirus type 5 (Ad5) vectors expressing a fragment of the gpS gene of porcine respiratory coronavirus. In the discussion section of this paper it states that the results of the study are in line with previous work conducted which shows that the maximum packaging capacity of the Ad5 virion is approximately 105% of the weight genome length since the authors attempted to make recombinants with larger inserts, all of which were unstable. Thus, it is submitted that there was no expectation that recombinant PAV would have a packaging capacity larger than the accepted rule.

Kleiboeker

Kleiboeker reports on the study of one porcine isolate NADC-1 and compares patterns of hybridization of NADC-1 fragments with human Ad2 and Ad5. There is no teaching of the construction of a porcine adenovirus vector and nothing more than a mere reference to PAV being a possible candidate for use as a viral vector. From the study it is concluded that there are regions of sequence similarity between NADC-1 and that of human Ad2 and Ad5. It is stated however that the putative E3 region of NADC-1 had considerably lower levels (30-35 %) of sequence similarity to human Ad 2.

The Examiner has stated that the porcine adenoviral E3 region shares common location and size to the E3 region of other adenoviruses and that the E3 region has been used in other adenoviruses for the insertion of heterologous sequences for protein expression *in vivo* and *in vitro*. The implication is that the E3 region of the PAV genome would be a suitable site for insertion. It is submitted that while the E3 region of PAV is in a similar location to other Mastadenoviruses, the size and nucleotide sequence are highly significantly different from human Ad 5 for which the majority of work had been conducted in regard to adenoviruses. Accordingly, Kleiboeker does not teach that the E3 region of PAV generally, would be a suitable site for insertion and again it does not teach the construction of any PAV vectors.

Reddy et al.

Reddy et al. reports on the nucleotide sequence analysis of the pVIII, E-3 and part of the fiber coding regions of porcine adenovirus serotypes 1, 2 and 3. The paper compares the sequence analysis of the E3 regions of porcine adenovirus serotypes 1, 2 and 3 and a putative E3 promoter but provides no functional data to demonstrate the activity of the putative promoter. The authors demonstrated that RNA transcripts from the E3 region were shorter than those from E3 regions of human adenoviruses. The paper merely proposes to use porcine adenoviruses 1, 2 and 3 as expression vectors; however, it provides no instruction or direction on how to construct a recombinant porcine adenovirus, no disclosure of any recombinants, nor any disclosure of the constructs of the invention. Further, Reddy et al. provides no information on the sequence or location of the major late promoter and tri-partite leader elements used in the recombinant porcine adenovirus constructs of the invention. Disclosure of the putative controlling elements discussed in Reddy et al. is insufficient to enable production of a recombinant porcine adenovirus and, more specifically, the production of the constructs of the invention.

The Examiner states that there would have been a motivation to use a porcine adenovirus vector for use as a vaccine in pigs as suggested by Kleiboeker or Reddy et al. since species specific adenoviruses will replicate at higher efficiency and result in a better vaccine. While it may have been expected that PAV in pigs would give a more species-specific vaccine vector, studies have shown however that that PAV replicates poorly in pigs and cannot be re-isolated from infected animals. Further, the findings of Torres et al. show that porcine cell lines allow human Ad5 to survive but do not categorically demonstrate human Ad5 replication and the production of *de novo* virus particles.

It is also submitted that the construction of transfer vector plasmids that bring together the major late promoter, tri-partite leader elements and polyadenylation signal, followed by the insertion of the foreign gene, transfection into a susceptible cell line and subsequent isolation and confirmation of a recombinant porcine adenovirus, requires a high degree of skill, expert knowledge and experimentation. Technical difficulties include, for example, a) Locating, isolating and cloning the major late promoter and tri-partite leader elements from the genome of the porcine adenovirus

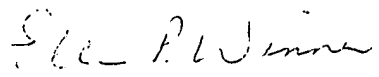
under study, b) Construction of transfer vector plasmids, c) Testing of suitable pig cells for susceptibility to PAV3 DNA transfection and subsequent virus infection, and d) Plaque purification of recombinant virus to ensure the absence of wild type virus.

In summary, none of the prior art documents cited are enabling disclosures since they provide no teaching on the production of recombinant porcine adenoviruses. The prior art does not suggest that PAV could package genomic lengths greater than 105% of the wild type. From the general knowledge in the art, the skilled person at the relevant time had no expectation that PAV could package genomic lengths greater than 105% of the wild type to produce stable recombinants. We submit that the teaching of the cited prior art cannot be extrapolated to PAV. Furthermore, even taking into account the combination of documents relied on by the Examiner, the invention is not obvious. Accordingly, reconsideration and withdrawal of the Examiner's rejections are respectfully requested.

CONCLUSION

In view of the foregoing arguments, this application appears to be in a condition for allowance. Passage to issuance is respectfully requested. It is believed that a fee of $400 for a two-month Extension of Time is due with this submission and a check in that amount of the Petition for Extension of Time are enclosed herewith. If the amount submitted is incorrect, please charge any underpayment or credit any overpayment to Deposit Account No. 07-1969.

Respectfully submitted,



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Docket No. 2-00

Marked changes
09/485,512 Amendment of 7/1/02

In the Specification:

Page 9, lines 25-26:

Figure [13] 11 graphically represents the percentage change in lymphocyte cell populations following vaccination with recombinant PAV-G-CSF.

Page 9, lines 27-28:

Figure [14] 12 graphically represents the change in stimulation of T-cells following vaccination with recombinant PAV-G-CSF.

Page 9, line 29:

Figures [15 a, b and c] 13A, 13B and 13C graphically illustrate a method of construction of a PAV E3 vector.

Page 16, lines 20-27:

In this experiment 5-6 week old piglets were used to represent immunocompetent pigs. A group of pigs (n=4) were vaccinated with recombinant PAV-G-CSF administered subcutaneously at a dose of 1 x 10^7 pfu per piglet. A second group (n=4) were vaccinated with PAV wild type (wt) administered subcutaneously at a dose of 1 x 10^7 pfu per piglet. A control group (n=4) were unvaccinated. Pigs were bled at 8 hour intervals for a period of 104 hours post vaccination. Complete blood counts were determined and the mean white blood cell (WBC) counts for each group monitored. These results are graphically represented in Figure 10 [and the percentage change in mean WBC counts graphically represented in Figure 11].

The paragraph bridging pages 17 and 18:

Differential WBC counts were also determined and monitored for each group. [The percentage change in mean monocyte cell populations graphically represented in Figure 12 and the percentage change in mean lymphocyte cell populations graphically

represented in Figure 13. Figure 12 shows that] The monocyte cell populations increased rapidly in pigs following vaccination with PAV wt, but were suppressed by vaccination with the recombinant PAV-G-CSF. This effect was due to the expression of G-CSF by the recombinant. A statistical analysis of these results is tabulated in Table 4. The analysis shows that there was a significant difference between the PAV wt and PAV-G-CSF from 32 to 96 hours post-vaccination. The percentage change in mean lymphocyte populations are graphically represented in Figure 11. Figure [13] 11 shows that there were shifts in lymphocyte cell population numbers following vaccination with the recombinant PAV-G-CSF. Unvaccinated controls show stable lymphocyte cell numbers over the duration of the experiment, whereas pigs vaccinated with PAV wt show a significant increase in lymphocyte cell population as a response to infection. Pigs vaccinated with the recombinant PAV-G-CSF show a decline in lymphocyte cell population. A statistical analysis of these results is tabulated in Table 5. The analysis shows that there was a significant difference between PAV wt and the recombinant PAV-G-CSF between 8 and 96 hours post vaccination. The different responses in lymphocyte cell proliferation following vaccination with recombinant PAV-G-CSF and PAV wt were due to the expression of G-CSF by the recombinant. These results show that vaccination with recombinant PAV-G-CSF produces a shift in sub-populations of cells involved in immunity.

The paragraph on page 19 beginning at line 8:

Figure [14] 12 graphically represents changes in the proliferation of T-cells of each group following stimulation with Concanavalin A (Con A). These results confirm that there was a significant proliferation of T-cells following vaccination with PAV wt at day 2 post vaccination, whereas vaccination with the recombinant PAV-G-CSF resulted in a suppression of T-cell proliferation by day 3.

In the Claims

31. (Once amended) A method of producing a recombinant porcine adenovirus vector for use as a vaccine including inserting into a non-essential region of a[n] porcine adenovirus genome, [at least one] a heterologous nucleotide

sequence in association with an effective promoter sequence to form a recombinant adenovirus vector greater than 105% the size of wild-type adenovirus.

39. (Once amended) A method of vaccination of pigs against disease including administering to said pigs a first recombinant porcine adenovirus vector stably incorporating, and capable of expression of [at least one] a heterologous nucleotide sequence encoding an antigenic determinant of said disease against which vaccination is desired, said adenovirus vector having a size greater than 105% the size of wild-type adenovirus.

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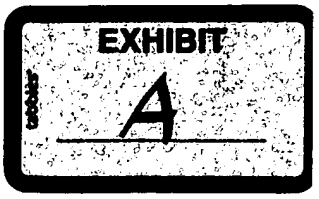

☐ 1: J Virol 1993 Oct;67(10):5911-21

Related Articles, Books, LinkOut

Packaging capacity and stability of human adenovirus type 5 vectors.

Bett AJ, Prevec L, Graham FL.

Department of Biology, McMaster University, Hamilton, Ontario, Canada.

Adenovirus vectors are extensively used for high-level expression of proteins in mammalian cells and are receiving increasing attention for their potential use as live recombinant vaccines and as transducing viruses for use in gene therapy. Although it is commonly argued that one of the chief advantages of adenovirus vectors is their relative stability, this has not been thoroughly investigated. To examine the genetic stability of adenovirus type 5 vectors and in particular to examine the relationship between genetic stability and genome size, adenovirus vectors were constructed with inserts of 4.88 (herpes simplex virus type 1 gB), 4.10 (herpes simplex virus type 1 gB), or 3.82 (LacZ) kb combined with a 1.88-kb E3 deletion or with a newly generated 2.69-kb E3 deletion. The net excess of DNA over the wild-type (wt) genome size ranged from 1.13 to 3.00 kb or 3.1 to 8.3%. Analysis of these vectors during serial passage in tissue culture revealed that when the size exceeded 105% of the wt genome length by approximately 1.2 kb (4.88-kb insert combined with a 1.88-kb deletion), the resulting vector grew very poorly and underwent rapid rearrangement, resulting in loss of the insert after only a few passages. In contrast, vectors with inserts resulting in viral DNA close to or less than a net genome size of 105% of that of the wt grew well and were relatively stable. In general, viruses with genomes only slightly above 105% of that of the wt were unstable and the rapidity with which rearrangement occurred correlated with the size of the insert. These findings suggest that there is a relatively tight constraint on the amount of DNA which can be packaged into virions and that exceeding the limit results in a sharply decreased rate of virus growth. The resultant strong selection for variants which have undergone rearrangement, generating smaller genomes, is manifested as genetic instability of the virus population.

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